“Evaluation of in vivo antioxidant potential of garlic derived phytochemical Allicin in different liver cancer cell lines”.

Synopsis

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1. **"Evaluation of in vivo antioxidant potential of garlic derived phytochemical Allicin in different liver cancer cell lines".**

2. INTRODUCTION:

In this era of rapid growth and industrialization the increase in the economy has by several ways invited several diseases and ailments which are caused due to multiple factors ranging from pollution, lifestyle, diet, drugs, microbes etc.

Diseases may be of different types and classification or may have some common as well as unique symptoms; it destroys the solidarity of an individual or a family (Shiv Kumar Yadav et al ,2020). Therapeutic measures taken against such ailments have also brought about several other health and wealth related complications. The advent of chemical therapeutic measures have not only caused additional side effects on the health but the measures have also caused a severe drainage of economy among the suffers. There has been an extensive research regarding the curative and preventive potencies of various targeted molecules of dietary plant origin like Genistein, Diazdin, Lycopene, Emodin, Allicin, Vanillium etc. These have shown great actions to cure dreaded diseases that either caused death or lead to several dreaded side effects when treated with synthetic drugs. These phytochemicals act through one or several signaling pathways and cobble not only to prevent but also to cure infectious diseases, parasite borne ailments, metabolic ailments, lifestyle related diseases and the most dreaded of all, cancer.

Cancer is a class of diseases characterized by out-of-control cell growth. Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). When a tumor successfully spreads to other parts of the body and grows,

invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis, and the result is a serious condition that is very difficult to treat. Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division and death. Western medicine does not have much to offer in terms of prevention apart from preventative mastectomies and questionable cervical cancer vaccines (vaccines pose dangers for the immune system).Once a cancer diagnosis is established modern medicine pulls out the heavy machinery Chemotherapy, radiation, surgery etc. The treatments are quite violent and invasive and its suspected that sometimes the treatment can kill the patient faster than the cancer would.

An estimated 19.3 million new cases and almost 10 million deaths have occurred from cancer in 2020 (Sung H et al 2021). Despite numerous research efforts in understanding cancer and in developing anticancer strategies, cancer remains a major lethal disease in people.

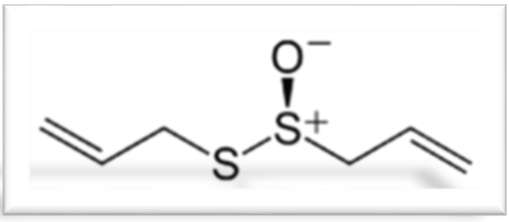
Reactive oxygen species (ROS), a class of highly bioactive molecules, have been widely studied in various types of cancers. Reactive oxygen species (ROS) have been extensively studied in various human diseases, including cancers. ROS are normal byproducts of a wide variety of cellular processes, including oxygen metabolism (Sena LA, Chandel NS 2012). The term "ROS" is a collective term referring to unstable, reactive, partially reduced oxygen derivatives that include hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and hydroxyl radical (•OH) (Cairns RA et al 2011).

These act as second messengers in cell signaling and are essential for various biological processes in normal and cancer cells (Chio IIC and Tuveson DA, 2017). Accumulating evidence suggests that ROS show activity that is a "double-edged sword" in cancer cells. At low to moderate levels, ROS act as signaling transducers to activate cancer cell proliferation, migration, invasion,

angiogenesis, and drug resistance (Wiseman H and Halliwell B. 1996). In other words, adequate levels of ROS are important for cancer cell homeostasis involved in the development of cellular processes such as proliferation, differentiation, migration, and cell death. In contrast, high levels of ROS are harmful to cancer cells and ultimately lead to cell death (Gorrini C, Harris IS, Mak TW, 2013). Accumulating evidence suggests that ROS show activity that is a "double-edged sword" in cancer cells. At low to moderate levels, ROS act as signaling transducers to activate cancer cell proliferation, migration, invasion, angiogenesis, and drug resistance (Wiseman H and Halliwell B, 1996),(Okon IS, Zou MH, 2015). In other words, adequate levels of ROS are important for cancer cell homeostasis involved in the development of cellular processes such as proliferation, differentiation, migration, and cell death. Antioxidants are usually considered to be beneficial for both cancer prevention and treatment as they can quench ROS levels, leading to a reduction in oxidative stress. The production of ROS is reported to be elevated in cancer cells as a result of increased metabolic rate, gene mutation, and relative hypoxia (Perillo B et al.2020).

The present piece of work aims to investigate the antioxidant activity of Allicin – garlic derived phytochemical on metabolism and progression of cancer cells in an artificial system which would throw light upon its use as an alternative therapeutic agent to substitute commercially available synthetic therapeutic agents against cancer with lots of painful side effects.

Structure of allicin



Preferred IUPAC name

2-Propene-1-sulfinothioic acid S-2-propenyl ester

Systematic name

3-[(Prop-2-ene-1-sulfinyl)sulfanyl]prop-1-ene

Properties	
Molecular formula	$C_6H_{10}OS_2$
Molar mass	162.27 g mol^{-1}
Appearance	Colourless liquid
Density	1.112 g cm^{-3}
Melting point	<25 °C
Boiling point	decomposes

The characteristic odor and flavor of garlic comes from sulfur compounds formed from allicin, the major precursor of garlic's bioactive compounds, which are formed when garlic bulbs are chopped, crushed, or damaged (Ross et al, 2006). Bioactive compounds are defined as substances in foods or dietary supplements, other than those needed to meet basic nutritional needs that are responsible for changes in health status.

Several population studies show an association between increased intake of garlic and reduced risk of certain cancers, including cancers of the stomach, colon, esophagus, pancreas, and breast. In China, frequent consumption of garlic and various types of onions and chives was associated with reduced risk of esophageal and stomach cancers, with greater risk reductions seen for higher levels of consumption (Gao, 1999 and Setiawan, 2005). Greater intake of allium vegetables (more than 10 g per day), particularly garlic and scallions, was associated with an approximately 50 percent reduction in prostate cancer risk (Hsing et *al,* 2002).

Allicin is the major biologically active component of garlic. First reported by Cavallito and Bailey (1944), allicin is the key ingredient responsible for the broad-spectrum of anti-bacterial activity in garlic. Research also showed that allicin is responsible for lipid-lowering, anti-blood

coagulation, anti-hypertension, anti-cancer, antioxidant and anti-microbial effects. Nonetheless, allicin is not found in fresh garlic.

The present research aims to investigate the antioxidant activity of allicin as a natural chemotherapeutic agent that could restrict the progression of cancer cells in an artificial system which would throw light upon its use as an alternative therapeutic agent to substitute commercially available synthetic therapeutic agents against cancer with lots of painful side effects.

PURPOSE OF RESEARCH:

- To determine enzymatic and non enzymatic antioxidant activity in cultured human cancer cell lines on Allicin treatment.
- To analyze the mechanism of induction of apoptosis in cancer cells on treatment with Allicin.
- To evaluate the effects of Allicin on oxidant induced damage of DNA in the cultured cell lines.
- To ascertain the potential of Allicin against cancer or itself can act as a good therapeutic agent cancer.

3. A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD:

Garlic (Allium *sativum*) is an herb that is used mainly as a food in many countries for its medicinal properties since ancient times. It enhances immune functions and has antibacterial, antifungal, antivirus and anticancer activities.

Anticancer properties of garlic were first described by Weisberger and Pensky (1958). They reported an inhibitory effect of a garlic extract on cancer cell growth both in vitro and in vivo.

Medicinal properties of garlic and other representatives of the family Allium (onion, shallot), including their anticancer efficacy, have been attributed to organosulfur compounds. The different health benefits of garlic are attributed to its sulfur-containing constituents.

These are classified as oil-soluble and water-soluble compounds. Oil-soluble compounds include diallyl sulfide, diallyl disulfide, and diallyl trisulfide, allyl methyl trisulfide, dithiins, and ajoene. The most important initial sulfur compound occurring in the intact garlic bulbs is alliin (S-allylcysteine sulfoxide). The whole bulbs contain also γ-glutamyl-S-allylcysteine, S-methylcysteine sulfoxide (methiin), Strans-1-propenylcysteine sulfoxide, S-2- (Amagase, 2006) . The reactions of allicin with -SH groups can yield S-allylcysteine or S-allylmercaptocysteine that is water soluble compounds (Rabinkov et al, 2000). Unlike oily sulfur compounds, water-soluble compounds are odorless and have more delicate and less characteristic flavor (Kodera et al, 2002). These compounds are also formed during aqueous garlic extraction, when the initial compound γ-glutamyl-S-allylcysteine is transformed into S-allylcysteine and this reaction is catalyzed by γ-glutamyltranspeptidase (γGT). S-allylcysteine along with its derivatives, S-methylcysteine and S-allylcysteine, are components of aqueous extracts of garlic and possess biological activity both in vitro and in vivo (Małgorzata et al, 2009).

Garlic compounds reduce serum lipid levels, as well as inhibit platelet aggregation. According to Lang et al (2004) allicin has radical scavenging properties in activated granulocytes and may inhibit inducible nitric oxide synthase expression in activated macrophages. Epidemiological studies and animal experiments have suggested that several garlic derived compounds have potential anticarcinogens (Sun and Wang, 2003), inhibit the proliferation of cancer cells and some of them cause apoptosis in tumor cells of different tissue origin (Kwon and Moon, 2005; Arditti and Rabinkov 2005; Jakubikova and Sedlak, 2006).

In a transformed hepatic cell culture model and in the rat mammary tumor model, treatment with garlic extracts with high selenium content resulted in growth inhibition, G1 phase cell cycle arrest and apoptotic DNA double strand breaks; there was significant cancer protection with the high selenium garlic, but none with regular garlic extract (Yamasaki and Lau, 1997). Whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalase and glutathione peroxidase (Ide and Lau, 1997; Popov et al, 1994).

Other in vitro studies show that Garlic extract and allicin efficiently scavenged exogenously generated hydroxyl radicals in a dose dependent fashion, but their effectiveness was reduced about 10% by heating to 100 degrees C for 20 minutes (Prasad et al, 1995; 1996). Other garlic constituents, such as S-allylcysteine, also demonstrated significant antioxidant effects in vitro (Imai et al, 1994). The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude, aged garlic extract (McCaleb,1993).

In vitro studies of Allicin's effect on human mammary MCF-7 cancer cells revealed that its antiproliferative activity is accompanied by accumulation of cells in the regulatory checkpoints, G0/G1 and G2/M, of the cell cycle (Hirsch et al, 2000). Interestingly, also other ally sulfur compound cause similar effects (Knowles and Milner, 2001).

The cause of apoptosis was related to decreased telomerase activity, an enzyme which allicin inhibits in a time- and dose- dependent manner (Sun and Wang, 2003). Allicin induces apoptosis in human cervical cancer SiHa cells and mouse fibroblast-like L-929 cells, manifested through the appearance of characteristic apoptotic morphological changes in apoptotic bodies, through DNA fragmentation, and activation of caspases 8, 9 and 3 (Oommen et al, 2004). Allicin was also shown to induce apoptosis in human epithelial carcinoma through a caspase-independent

pathway, mediated by the release of apoptotic-inducing factor (AIF) from mitochondria and protein kinase A (PKA) activation (Park et al, 2005). More results describing the effects of allicin or other garlic derieved products on various proteins participate in the apoptotic process have already been reported (Antlsperger et al, 2003). Several in vivo studies indicate that garlic and sulfur containing garlic compounds suppress the incidence of tumors in rodent models (Liu et al, 1992; Amagase and Milner, 1993; Song and Milner, 1999). Studies also indicate that sulfur-containing garlic compounds have anti-mutagenesis and anti-carcinogenesis effects (Milner, 1996). Numerous pharmacological studies have demonstrated that garlic has many beneficial health effects (Steiner and Lin, 1998; Rahman, 2007). Epidemiological studies, preclinical investigations and clinical trials support the role of garlic compounds as potent cancer chemo preventive agents. A recent report indicates that allicin (a biological active compound of garlic) induces mitochondrial release of cytochrome c, activation of caspase 9 and 3 and DNA fragmentation in U937 cells in a concentration-and time-dependent manner (Miron et al, 2008). Although it is known that oxidative insults to DNA can lead to mutations in crucial genes, which may ultimately lead to cancer (Ames et al, 1993), one of the molecular mechanisms by which bioactive compounds induce cytotoxicity and apoptosis is through oxidative stress. Many studies have shown that garlic possesses antioxidant and anti-lipid peroxidative effects (Borek, 2001).

4. PROPOSED METHODOLOGY IN BRIEF:

The study would encompass treatment of Allicin to cultured liver cancer cell lines and analysis of various parameters in comparison to untreated control.

A. Antioxidant Analysis

i. Enzymatic antioxidant assay

ii. Non enzymatic antioxidant assay

B. Cytotoxicity Assay

C. Biochemical Assays

D. Statistical Analysis of results would be done using a suitable test of significance.

5. EXPECTED OUTCOME OF THE PROPOSED WORK:.

The study would help in understanding the antioxidant potential of Allicin which would be a prelude for understanding the mechanisms of interaction of Allicin with cellular molecules like DNA and proteins which are the key factors responsible for regulation of cell cycle and progression of cancer. The study would facilitate in designing the alternative drugs and to decide accurate delivery systems to treat cancer. The present study would establish allicin as a candidate drug in the treatment of cancer.

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